Journal of Pioneering Medical Sciences
Received: January 23, 2025 | Accepted: March 13, 2025 | Published: April 27, 2025
Volume 14, Issue S01, Pages 278-282

DOI https://doi.org/10.47310/jpms202514S0135

In Vitro Evaluation of Antifungal Activity of a Dental Varnish Formulated with Ginger and Rosemary Mediated Titanium Oxide Nanoparticles

Bhavesh Sureendar[1] and Lalitha Rani Chellappa[2]

[1]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[2]Department of Public Health Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Author Designation: [1]Student, [2]Assistant Professor

*Corresponding author: Lalitha Rani Chellappa (e-mail: lalitharanibds28@gmail.com).

Abstract **Background:** The increasing prevalence of fungal infections like *Candida albicans* in the oral cavity has highlighted the need for alternative antifungal agents. Titanium oxide nanoparticles (TiO_2 NPs), synthesized through green methods using plant extracts, offer promising antimicrobial properties. This in vitro study investigates the antifungal potential of a dental varnish formulated with ginger and rosemary-mediated TiO_2 nanoparticles against *C. albicans*. **Materials and Methods:** Mueller Hinton agar plates were prepared and inoculated with *Candida albicans*. Using the agar well diffusion method, wells (9 mm) were filled with varying concentrations of the prepared dental varnish (25 µg/mL, 50 µg/mL, 100 µg/mL). A commercial dental varnish and fluconazole served as control groups. Plates were incubated at 37 °C for 24-48 hours. Zones of inhibition were measured in millimetres, and optical density was assessed using a spectrophotometer at various time intervals to evaluate fungal growth kinetics. **Results:** The antifungal activity of the nanoparticle-based varnish increased with concentration. At 25 µg/mL, 50 µg/mL, and 100 µg/mL, the zones of inhibition measured 18 mm, 22 mm, and 30 mm, respectively. The commercial dental varnish produced a 20 mm zone of inhibition. A notable reduction in optical density was observed with higher concentrations over 5 hours, confirming effective fungal suppression. **Conclusion:** The ginger and rosemary-mediated TiO_2 NP-based dental varnish demonstrated significant in vitro antifungal activity against *Candida albicans*. These findings suggest its potential use in preventive dental care, especially for patients susceptible to fungal colonization. Further studies involving biofilm models and clinical trials are recommended to validate its efficacy and safety in real-world conditions.

Key Words TiO_2 nanoparticles, *Candida albicans*, dental varnish, antifungal activity, rosemary, ginger, *in vitro* study

INTRODUCTION

Over the past decade, nanotechnology has significantly advanced and become an integral part of medical and dental sciences due to its wide-ranging applications in diagnostics, drug delivery and antimicrobial treatments. Among these, metal nanoparticles have garnered particular interest for their potent antibacterial and antifungal properties [1]. In recent years, there has been a shift toward green synthesis of nanoparticles to minimize the use of hazardous chemicals and enhance biocompatibility. This environmentally friendly approach often involves biological sources such as plants, fungi, bacteria and algae, which act as reducing and stabilizing agents in nanoparticle formation [2,3].

Among inorganic nanoparticles, titanium dioxide (TiO_2) stands out due to its large-scale production, high biocompatibility, low toxicity to human cells and strong antimicrobial effects. TiO_2 nanoparticles can be synthesized via physical, chemical, biological, or green methods, with the latter gaining popularity for being non-toxic and eco-friendly [4]. The use of herbal or plant-based agents, such as ginger, clove and rosemary, has proven to be a cost-effective, rapid and sustainable route to nanoparticle synthesis [5,6]. These plant-mediated methods avoid hazardous byproducts and leverage the inherent antimicrobial properties of phytochemicals.

There has been growing interest in nanoparticle applications within dentistry, particularly for the management of oral biofilm-associated diseases like dental caries and periodontal infections. Dental caries is initiated by acidogenic bacteria, notably Streptococcus mutans and is often worsened by opportunistic pathogens such as *Candida albicans*, which can form mixed biofilms and contribute to enamel and dentin demineralization [7-9].

Previous research has documented the synthesis and characterization of various nanoparticles including halloysite nanotubes, zirconium oxide, selenium, chitosan nanocomposites, nanoemulsions and plant-derived silver NPs [7,10]. TiO_2 nanoparticles, in particular, have demonstrated efficacy against multidrug-resistant microbes, reinforcing their value in therapeutic dental applications [10,11].

Ginger and rosemary, both widely known for their antimicrobial, anti-inflammatory and antioxidant properties, contain active compounds such as gingerol and rosmarinic acid, which not only assist in reducing metal ions during nanoparticle synthesis but also enhance the antimicrobial efficacy of the resulting nanomaterials.

Despite the known potential of green-synthesized TiO_2 NPs, limited studies have explored their integration into dental varnish formulations for direct antifungal application, especially targeting *Candida albicans*. This gap forms the basis of the current study.

The aim of this study is to evaluate the antifungal activity of a dental varnish formulated using ginger and rosemary-mediated TiO_2 nanoparticles against *C. albicans* under *in vitro* conditions.

MATERIALS AND METHODS

Study Setting

This *in vitro* experimental study was conducted in the Nanomedicine Laboratory, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India. The oral pathogen *Candida albicans* was selected for antifungal testing. The microbial strain was sourced from the microbiological culture collection of the same institution.

Antifungal Activity Assessment

The antifungal activity of the ginger and rosemary-mediated titanium dioxide nanoparticles (TiO_2 NPs) was evaluated using the agar well diffusion method. Mueller Hinton Agar (MHA) plates were prepared and sterilized in an autoclave at 121°C for 15-20 minutes. The sterilized medium was poured into sterile Petri dishes and allowed to solidify at room temperature.

A standardized suspension of *Candida albicans* was prepared and 100 µL of the culture was evenly spread across the surface of each agar plate using sterile cotton swabs. Wells of 9 mm diameter were created in the agar using a sterile polystyrene tip. These wells were then loaded with three different concentrations of TiO_2 NPs-based dental varnish: 25 µg/mL, 50 µg/mL and 100 µg/mL. Each test was performed in triplicate to ensure reproducibility.

Fluconazole (standard antifungal agent) was used as a positive control. The plates were incubated at 37°C for 24 and 48 hours. After incubation, the zone of inhibition surrounding each well was measured in millimetres (mm) using a transparent ruler. The average diameter from three measurements per well was recorded and the antifungal activity was expressed as the mean zone of inhibition.

Preparation of Plant-Mediated TiO_2 Nanoparticles for Dental Varnish

To formulate the nanoparticle-based dental varnish, 6.26 g of TiO_2 powder was dissolved in 60 mL of distilled water and mixed with 40 mL of ginger and rosemary plant extract in an orbital shaker. The mixture was monitored for colour change at 2-hour intervals using ultraviolet-visible (UV-Vis) spectroscopy, indicating nanoparticle synthesis.

After 36 hours of continuous shaking, the solution underwent centrifugation at 7,000 rpm for 10 minutes to isolate the TiO_2 nanoparticles. The resulting pellet, consisting of ginger and rosemary-mediated TiO_2 NPs, was collected and stored as a stock solution. This stock was subsequently diluted to prepare working concentrations for use in the antifungal assay.

RESULTS

The antifungal efficacy of the ginger and rosemary-mediated TiO_2 nanoparticle-based dental varnish was found to be concentration-dependent. As the concentration of the nanoparticles increased from 25 µg/mL to 100 µg/mL, a corresponding increase in the zone of inhibition against *Candida albicans* was observed (Figure 1, Table 1). The mean zones of inhibition were recorded as follows:

- 25 µg/mL: 18 mm
- 50 µg/mL: 22 mm
- 100 µg/mL: 30 mm

By comparison, the commercial dental varnish demonstrated a 20 mm zone of inhibition and fluconazole (used as the positive control) produced a zone of 32 mm (Figure 2).

Additionally, optical density (OD) values measured at regular time intervals (1 to 5 hours) showed a progressive decline in fungal growth with increasing TiO_2 NP concentrations. At 100 µg/mL, the most significant reduction in OD was observed by the 5th hour, indicating strong antifungal kinetics (Figure 3).

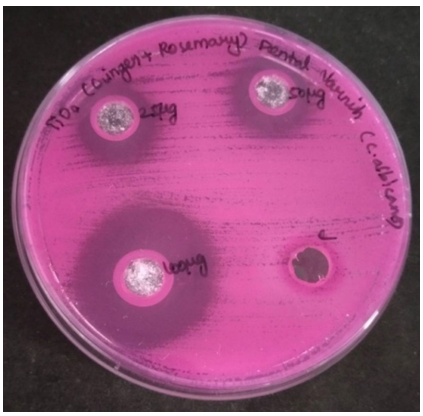

Figure 1: The above figure represents the culture plate of *C. albicans* in which dental varnish was used to find out the zone of inhibition

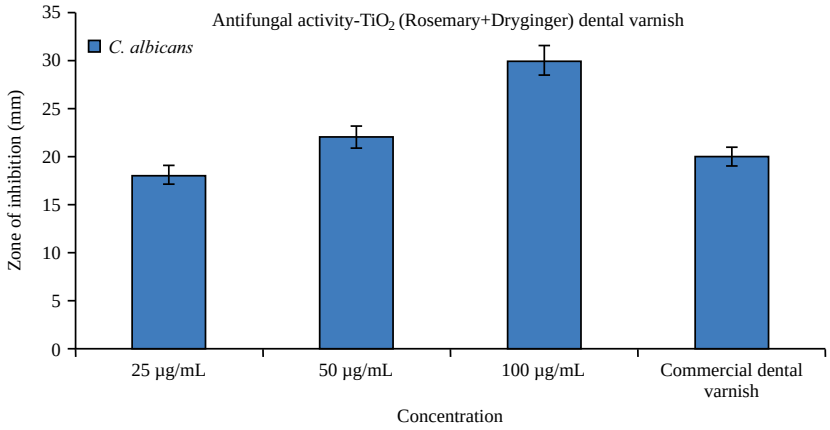

Figure 2: The figure shows the zone of inhibition of the commercial dental varnish prepared against *Candida albicans*, The X-axis represents the concentration of the sample, Y-axis represents the zone of inhibition in mm

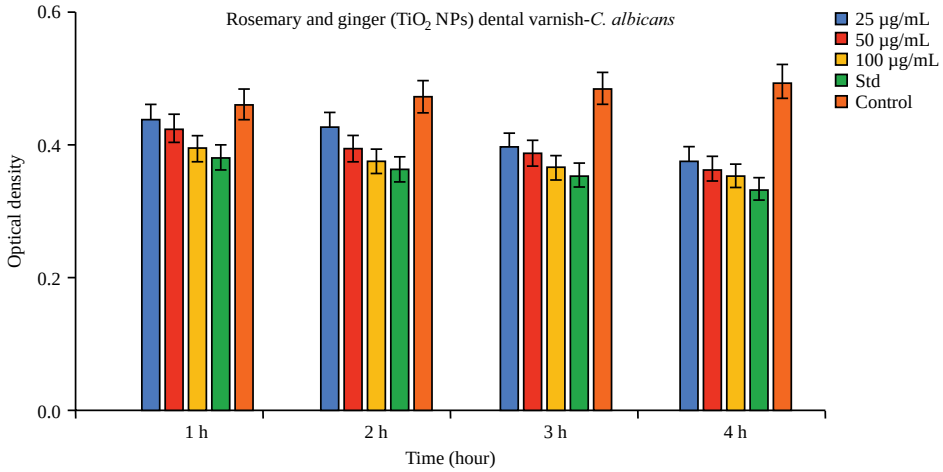

Figure 3: The figure shows the time kill curve assay of *C. albicans*. It shows the antifungal activity of rosemary and ginger-mediated TO, NPs-based dental varnish against *S. aureus* species tested at various concentrations from 25, 50, 100 ML, at various time intervals from 1 hr, 2 hr, 3 hr, 4 hr, 5 hr and the percentage of inhibition, the X-axis shows the time interval of the NPs and the Y-axis shows the optical density

Table 1: The above table represents the value of zone of inhibition of the prepared dental varnish against *C. albicans*

Organism	25 µg/mL	50 µg/mL	100 µg/mL	Commercial dental varnish
C. albicans	18	22	30	20

The experimental varnish also exhibited notable antimicrobial activity against Streptococcus mutans and Enterococcus faecalis when tested under similar conditions, although the primary focus of this study remained on antifungal effects against *C. albicans*.

These results collectively demonstrate that the green-synthesized TiO_2 NP dental varnish possesses potent antifungal activity, particularly at higher concentrations and could be a promising candidate for preventive oral healthcare applications.

DISCUSSION

Dental caries remains one of the most prevalent oral health conditions globally, with a high incidence and significant potential to cause irreversible damage if left untreated. Caries development begins with the formation of a biofilm on the tooth surface, triggered by various intra- and extraoral stimuli. In response, numerous preventive strategies have emerged, including the integration of antimicrobial agents into dental materials to inhibit biofilm formation and progression.

Previous studies, demonstrated that calcium silica nanoparticles effectively prevented enamel erosion, underlining the potential of nanotechnology in preventive dentistry [12,13]. Similarly, sudy evaluated a dental varnish incorporating TiO_2 nanoparticles (NPs) synthesized using ginger and clove, showing substantial antibacterial activity against caries-causing microbes [14,15]. These findings are consistent with the present study, which used ginger and rosemary-mediated TiO_2 NPs and demonstrated significant antifungal activity against *Candida albicans*.

The incorporation of plant-derived TiO_2 NPs into dental materials is gaining traction due to their broad-spectrum antimicrobial properties and eco-friendly synthesis routes. Recent investigations have shown that TiO_2 NPs exhibit antibacterial and antibiofilm activity against both gram-positive and gram-negative pathogens, particularly Streptococcus mutans and Lactobacillus species, which play a critical role in the initiation and progression of carious lesions [16]. The current formulation builds on these findings by also targeting fungal pathogens, specifically *C. albicans*, a frequent contributor to oral infections and denture-related stomatitis.

The photocatalytic activity, UV absorption capability and ROS-generating properties of TiO_2 NPs make them a versatile component in dental composites and adhesives. These nanoparticles function by producing reactive oxygen species (ROS) under light exposure, leading to microbial membrane disruption and cell death [17]. This aligns with the present study's findings, which showed a concentration-dependent reduction in *C. albicans* growth, likely mediated by such oxidative mechanisms.

Further, acid-functionalized TiO_2 NPs, as discussed by Alofi et al., have demonstrated potential for integration into dental resins, enhancing not only antimicrobial effects but also mechanical properties and adhesion to tooth surfaces [18]. In our study, the green-synthesized nanoparticles also exhibited uniform dispersion, a critical factor in achieving consistent antimicrobial coverage in dental varnish formulations.

In addition to antimicrobial efficacy, the morphological compatibility of TiO_2 NPs with polymer matrices improves the mechanical stability and durability of the composite. Studies have shown that the inclusion of TiO_2 as a reinforcing agent leads to improved interfacial adhesion and dispersion, which in turn enhances the varnish's structural performance. This suggests that TiO_2 NPs are not only biologically active but also mechanically synergistic with resin materials [19].

Finally, the present study underscores the antifungal capabilities of the synthesized dental varnish, which may extend the application of nanoparticle-based formulations beyond bacterial control to address fungal infections in high-risk dental populations. The targeting of *Candida albicans* is particularly significant, given its role in denture stomatitis and oral candidiasis in immunocompromised individuals.

Overall, these findings contribute to the expanding body of evidence supporting the therapeutic versatility of green-synthesized TiO_2 nanoparticles, especially when reinforced with herbal extracts. The integration of ginger and rosemary, both known for their bioactive phytochemicals, enhances the antifungal potential while maintaining biocompatibility and sustainability.

CONCLUSION

This *in vitro* study demonstrated that a dental varnish formulated with ginger and rosemary-mediated titanium dioxide nanoparticles (TiO_2 NPs) exhibited significant antifungal activity against *Candida albicans*. The results support the potential application of this green-synthesized formulation as a biocompatible and eco-friendly antifungal agent in preventive dental care. With further validation, including studies on tissue compatibility, toxicity and long-term stability, this novel dental varnish may serve as a viable adjunct in managing oral fungal infections, especially in high-risk populations.

Limitations

This investigation was limited to an *In Vitro* experimental setting using a small number of test samples. As such, while the antifungal effects were promising, these findings may not fully reflect real-world therapeutic outcomes. The absence of biofilm models, cytotoxicity assessments and mechanical

stability tests limits the ability to generalize these results to clinical settings. Future research should include ex vivo studies, biofilm inhibition assays and clinical trials to determine the mechanism of action, long-term efficacy and safety profile of the TiO_2 NP-based dental varnish in oral healthcare applications.

Ethical Consideration

This study was conducted using *In Vitro* experimental methods and did not involve human or animal subjects. Therefore, ethical approval was not required. All laboratory protocols were carried out in accordance with institutional biosafety and research guidelines.

Conflict of Interest

The authors declare no conflict of interest related to this study.

Acknowledgement

The authors sincerely thank the Department of Pharmacology and the Nanomedicine Laboratory at Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, for providing infrastructure and support to carry out this research.

REFERENCES

[1] Chokkattu, Jerry Joe, *et al.* "Embryonic toxicology evaluation of ginger- and clove-mediated titanium oxide nanoparticles-based dental varnish with zebrafish." *The Journal of Contemporary Dental Practice*, vol. 23, no. 11, November 2022, pp. 1157-1162. https://pubmed.ncbi.nlm.nih.gov/37073941/.

[2] Li, Yilan, *et al.* "Titanium dioxide nanoparticles enhance photocurrent generation of cyanobacteria." *Biochemical and Biophysical Research Communications,* vol. 672, September 2023, pp. 113-119.

[3] Mathew T. Maliael, *et al.* "Antibacterial Activity of Nanoparticle-Coated Orthodontic ArchwiresA Systematic Review." *Journal of International Oral Health,* vol. 15, no. 1, February 2023, pp. 1-7. https://journals.lww.com/jioh/fulltext/2023/15010/antibacterial_activity_of_nanoparticle_coated.1.aspx.

[4] Wu, Yanyan,*et al.* "Adverse effects of titanium dioxide nanoparticles on beneficial gut bacteria and host health based on untargeted metabolomics analysis." *Environmental Research,* vol. 228, July 2023. https://www.sciencedirect.com/science/article/abs/pii/S0013935123007132.

[5] Elazab, Mahmoud A.,*et al.* "Effect of Dietary Rosemary and Ginger Essential Oils on the Growth Performance, Feed Utilization, Meat Nutritive Value, Blood Biochemicals, and Redox Status of Growing NZW Rabbits." *Animals,* vol. 12, no. 3, February 2022. https://pubmed.ncbi.nlm.nih.gov/35158698/.

[6] Missier, M., *et al.* "Antibacterial Properties of Lemon Juice–Mediated Zinc Oxide Nanoparticle and Titanium Dioxide Nanoparticle." *Journal of Pharmacy and Bioallied Sciences,* vol. 16, no. Suppl 5, December 2024, pp. S4421-S4425. https://www.semanticscholar.org/paper/Antibacterial-Properties-of-Lemon-Juice%E2%80%93Mediated-Missier-Ramakrishnan/1e2aead2ccd598b475712624924886e79823d1a6.

[7] Ezhilarasan, Devaraj and Karthik Shree Harini. "Nanodrug delivery: Strategies to circumvent nanoparticle trafficking by Kupffer cells in the liver." *Journal of Drug Delivery Science and Technology,* vol. 86, September 2023. https://www.sciencedirect.com/science/article/abs/pii/S177322472300583X.

[8] Romoli, Jéssica Cristina Zoratto, *et al.* "Anti-mycotoxigenic and antifungal activity of ginger, turmeric, thyme and rosemary essential oils in deoxynivalenol (DON) and zearalenone (ZEA) producing Fusarium graminearum." *Food Additives & Contaminants,* vol. 39, no. 2, December 2021, pp. 362-372. https://repositorio.usp.br/item/003055616.

[9] Ilancheran, Priyan, *et al.* "Green synthesis, characterization, and evaluation of the antimicrobial properties and compressive strength of hydroxyapatite nanoparticle-incorporated glass ionomer cement." *Cureus,* vol. 16, no. 4, April 2024. https://pubmed.ncbi.nlm.nih.gov/38770461/.

[10] Yuvaraj, Ashna, *et al.* "Anti-Inflammatory and Antifungal Activity of Zinc Oxide Nanoparticle Using Red Sandalwood Extract." *Pharmacophore,* vol. 14, no. 1, January 2023, pp. 25-31.

[11] Gracias, Slavika, *et al.* "Fabrication of chitosan nanocomposites loaded with biosynthetic metallic nanoparticles and their therapeutic investigation." *Environmental research,* vol. 234, October 2023. https://pubmed.ncbi.nlm.nih.gov/37437861/.

[12] Zarrabian, Shirin. *Development of improved slow-release dental varnish formulations effective against Streptococcus mutans in vitro.* https://openlibrary.org/books/OL17063324M/Development_of_improved_slow-release_dental_varnish_formulations_effective_against_Streptococcus_mut.

[13] Tavares, Fernanda Michel, *et al.* "The use of a new calcium mesoporous silica nanoparticle versus calcium and/or fluoride products in reducing the progression of dental erosion." *Journal of applied oral science,* vol. 28, July 2020. https://pmc.ncbi.nlm.nih.gov/articles/PMC7384487/.

[14] Selvi, V.T. Thamarai, *et al.* "Green Synthesis of Titanium Oxide Nanoparticles With Rosemary and Ginger and Their Bactericidal Action Against Staphylococcus aureus." *Cureus,* vol. 15, no. 9, September 2023. https://pubmed.ncbi.nlm.nih.gov/37885543/.

[15] Nasim, Iffat, *et al.* "Effect of nanoparticle based intra-canal medicaments on root dentin micro-hardness." *Bioinformation,* vol. 18, no. 3, March 2022, pp. 226-230. https://pubmed.ncbi.nlm.nih.gov/36518131/.

[16] Tayyeb, Jehad Zuhair, *et al.* "Multifunctional curcumin mediated zinc oxide nanoparticle enhancing biofilm inhibition and targeting apoptotic specific pathway in oral squamous carcinoma cells." *Molecular biology reports,* vol. 51, no. 1, March 2024. https://pubmed.ncbi.nlm.nih.gov/38489102/.

[17] Wu, Aiguo and Wenzhi Ren. *TiO2 Nanoparticles: Applications in Nanobiotechnology and Nanomedicine* United states, John Wiley & Sons, ISBN-17: 978-3-527-82544-8, Pages: 256. https://www.wiley.com/en-us/TiO2+Nanoparticles%3A+Applications+in+Nanobiotechnology+and+Nanomedicine-p-9783527825448.

[18] Alofi, Saleh, *et al.* "Study and Modeling of the Kinetics of the Photocatalytic Destruction of Stearic Acid Islands on TiO2 Films." *The Journal of Physical Chemistry,* vol. 127, no. 25, June 2023, pp. 12194-12205. https://pubs.acs.org/doi/10.1021/acs.jpcc.3c00952.

[19] Do, Trong-On and Sakar Mohan. *Emerging Trends in TiO2 Photocatalysis and Applications* Switzerland, Catalysts, ISBN-17: 978-3-03936-707-8, Pages: 596. https://www.mdpi.com/books/reprint/2638-emerging-trends-in-tio2-photocatalysis-and-applications.